SNARe™ Whole Blood Genomic DNA Purification 96-Well Microtiter Plate Protocol

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BEADS ABOVE THE REST™

Description

Mammalian Genomic DNA may be isolated and purified using the SNARe Whole Blood Genomic DNA Purification System. The microtiter plate format is a high throughput technique amenable to manual and/or automated system applications.

Automated microtiter plate procedures generally take between 15-45 minutes per plate, with an average yield of 5-20µg DNA per well, depending upon the starting sample volume.

Material

Material Required

- Multi-well precision pipets with disposable tips to deliver 1-20µL, 20-200µL, 200-1000µL
- Centrifuge with carriers equipped for 96-well plates, capable of 1000 X G (optional)
- 1 x 96-well polypropylene microtiter plate preferably with round or U-bottom, capable of holding 200-500µL of fluid volume. (Example: QIAGEN® or NUNC™)
- 1-8 x 96-deep well polypropylene plate(s), preferably with round or U-bottom, capable of holding 1-2mL of fluid volume. (Example: QIAGEN or NUNC)
- EDTA, ACD, or Heparin treated whole blood or buffy coat, purified white blood cells or mononuclear cell preparations.
- 96-well plate magnetic separator (for use with manual sample processing)
- Tween® 20 (optional)
- Microtiter plate mixer (manual sample processing)
- Plate sealer

Procedure

Researchers are advised to optimize the use of particles, reagents, and disposable equipment for their specific applications.

Preparation of DNA Separation Particles

(For procedure, see Product Data Sheet 691, under the ‘Procedures’ section on Page 2.)

Scale up the volume of particles required in order to perform multiple tests using a 96-well microtiter plate.

Preparing Samples

(For procedure, see Product Data Sheet 691, under the ‘Procedures’ section on Page 3.)

Be sure the whole blood is at room temperature and is well mixed prior to use. When using frozen whole blood, thaw the sample quickly and mix well prior to dispensing into wells of a microtiter plate (ex. 100µL/well). Using the volumes in Step 7 as a guide, larger whole blood volumes can be lysed. Scale up the reagents required to match the whole blood volume in a 15mL or 50mL centrifuge tube, in order to use more of the wells in a 96-well microtiter plate assay. To ensure efficient lysis, be sure to mix the blood sample and the lysis buffer well by pipetting up and down several times, while at the same time avoiding formation of excess bubbles or foaming. Generally, 100µL blood samples are lysed in 20µL of Proteinase K (8602436) and 100-400µL of Whole Blood Lysis Buffer, then heated in a 50-70˚C water bath for approximately 10 minutes, with occasional mixing.

Binding, Washing and Elution of the DNA – (Automated Protocol)
(Example uses the QIAGEN BioSprint™ 96 automated sample processing system, in which the magnetic particles are transferred from one microtiter plate, containing reagent, to the next.)

1. Fill deep well microtiter plates (2-8) with the following volumes of each reagent:
   - 100µL of lysate (Fresh or frozen whole blood, WBCs, or MNCs)
   - 100µL/well Ethanol
   - 50µL of DNA Binding Buffer
   - 20µL of washed DNA Separation particles

Slot Position	*Reagent*	*Volume (µL)*
Slot 8	Lysate with Ethanol, DNA Binding Buffer, and particles	270-690µL
Slot 7	Wash #1 DNA Wash Solution	500-1000µL
Slot 6	Wash #2 DNA Wash Solution	500µL
Slot 5	Wash #3 Ethanol 70%	500µL
Slot 4	Wash #4 Ethanol 70%	500µL
Slot 3	RNase free water + 0.02% Tween 20*	500µL
Slot 2	DNA Elution Buffer	50-200µL**
Slot 1	Empty 300-500µL Microtiter Plate with 96-Magnetic rod cover	NA

* The addition of RNase water with 0.02% Tween 20 is optional, and may assist in removing loosely bound protein.
** The volume of DNA Elution Buffer can vary to optimize DNA concentration, depending upon the user's requirements.

2. Place each of the microtiter plates onto the BioSprint 96 carousel in each specified slot, and initiate the "DNA Blood 100" program. This program takes approximately 15 minutes to process the lysate.

Binding, Washing, and Elution of the DNA – (Manual Protocol)
(Example uses BioMag® side pull or bottom pull separators, in which the magnetic particles remain in each microtiter plate well, with the addition of each reagent to each well separately.)

1. Using a **96 deep well microtiter plate** add the following:
   - 100µL of lysate (Fresh or frozen whole blood, WBCs, or MNCs)
   - 100µL/well Ethanol
   - 50µL of DNA Binding Buffer
   - 20µL of washed DNA Separation Particles
2. Incubate the plate with mixing at room temperature for 10 minutes.
3. Place the plate on the magnetic separator until the supernatant is clear. Then with the plate on the separator, remove and discard the supernatant.
4. Add 1.0mL of DNA Wash Solution to each well. Mix using pipette mixing or plate mixer.
5. Place the microtiter plate onto the plate magnetic separator until the supernatant is clear, then remove and discard the supernatant.
6. Repeat the wash step with 0.5mL of DNA Wash Solution to each well.
7. Place the microtiter plate onto the plate magnetic separator until the supernatant is clear, then remove and discard the supernatant.
8. Wash the particles with 0.5mL of 70% EtOH. Resuspend the DNA-particle complex by pipette mixing or by using a plate mixer.
9. Place the microtiter plate onto the plate magnetic separator until the supernatant is clear, then remove and discard the supernatant.
10. Repeat the wash step with 0.5mL of 70% EtOH. Resuspend the DNA-particle complex by pipette mixing or by using a plate mixer. Place the microtiter plate onto the plate magnetic separator until the supernatant is clear, then remove and discard the supernatant.
11. After removing the residual EtOH, air dry the particle-DNA complex for 2-5 minutes.
12. Using the DNA Elution Buffer, elute the bound DNA by resuspending the particle in a volume between 50-200µL, using pipette mixing.
13. Seal and incubate the microtiter plate at room temperature or at 50˚C for 10 minutes.
14. Place the microtiter plate on the magnetic separator until the supernatant clears.
15. Carefully remove the eluate and transfer to another clean microtiter plate or test tube for further applications.

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